EXPERIMENTS TO EXAMINE TRANSPLANT PROCEDURES ON THE

SEAGRASS

HALODULE BEAUDETTEI

A Thesis

by

FREDERICK JOSEPH LAND

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Rangeland Ecology and Management

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Approved by:

Chair of Committee, James W. Webb
Committee Members, Fred Smeins
Richard Fisher
Head of Department, Steven Whisenant

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ABSTRACT

Experiments to Examine Transplant Procedures on the Seagrass

Halodule beaudettei. (May 2006)

Frederick Joseph Land, B.S., Texas A&M University

Chair of Advisory Committee: Dr. James W. Webb

During the growing seasons of 1999 and 2000 five experiments were performed to test growth of the seagrass *Halodule beaudettei* (shoal-grass) in nursery pond conditions. Sediment oxidation state, sediment source, container type, flow regime, and light transmittance were tested to improve nursery pond cultivation techniques and to test assumptions about the decline of seagrasses in Galveston Bay, Texas. Oxidized and reduced sediments exhibited no statistical difference as mean percent change in the number of stems of shoal-grass, after 47 days. Sediment from three source locations, West Bay, East Beach Lagoons, and the experimental pond bottoms, showed no significant difference in the mean percent change in the number of stems of shoal-grass at 48 and 95 days. A statistical difference was seen in the container type experiment, trays versus pots, at 48 days where shoal-grass had double the number of stems produced in trays; however no significant difference was found at 93 days. A significant difference was found in the flow regime experiment, no-flow versus flow, at 47 days in the mean percent change of shoal-grass with double the number of stems produced in the flow regime. Significant differences were observed between the low light and high light treatments with shoal-grass, widgeongrass

(*Ruppia maritima*), star grass (*Halophila engelmannii*), and turtlegrass (*Thalassia testudinum*), with survival and growth occurring in the high light treatment and decline and death occurring in the low light treatment. The importance of reduced sediment may have been overstated in the past as sediment reduction occurs rapidly with submersion. It appears that while West Bay sediment did not have a deleterious effect on shoal-grass, West Bay simulated light conditions did. Container type seems to be important at first but not so much in the long term. Some flow, water movement, or current appears to be important.

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INTRODUCTION

There are more than fifty species of seagrasses worldwide (Fonseca, 1989; den Hartog, 1977). Six of those species are native to the coastal areas of Texas (Fonseca, 1994) and belong to two families, Cymodoceaceae and Hydrocharitaceae (Jones et al., 1997). *Ruppia maritima* (widgeon-grass) which belongs to the family Ruppiaceae, though not considered a true seagrass, is often found growing with other seagrasses (Kantrud, 1991).

Seagrasses are the basis of one of the most productive habitats on earth (Fonseca, 1994). A healthy bed of seagrass produces tremendous biomass that provides surface area for the growth of epiphytic algae. These algae, along with seagrasses, can be an important base to the estuarine food chain. Many commercially important species of crabs, shrimp, and fish utilize seagrass habitat, and it is widely accepted that their numbers would be greatly reduced without it (Hammerstrom et al., 1998; Dunton, 1996; Short and Wyllie-Echeverria, 1996; Sheridan, 1992). According to Chambers (1992) ninety-eight percent of commercially important species from the U.S. Gulf Coast spend some part of their life cycle in seagrass habitat. Ducks, sea turtles, and manatees also consume seagrasses making this a unique habitat that is vital to many marine species (Ferguson and Korfmacher, 1997; McMahan, 1970; Cottam et al., 1944).

Seagrass meadows are important and beneficial since they slow erosion by buffering wave action and hold sediments in place.

This thesis follows the style and format of the journal Wetlands.

As the wave energy is reduced, suspended particles fall out and become trapped among the grass blades, rhizomes, and roots (Fonseca, 1989). Seagrass meadows not only increase sediment stabilization, but they also provide positive feedback in water clarity and photosynthetic output.

When damaged, seagrass beds lose the ability to hold sediments, water conditions degrade, and photosynthetic efficiency declines (Vermaat et al., 1996). With persistent disturbance, the seagrass may die and the habitat may be lost. Further, a number of natural disturbances including tropical storms, hurricanes, shifting underwater channels, seasonally extreme tide levels (high and low), overgrazing, burrowing animals (sting rays, crabs, etc.), and diseases can cause severe damage to seagrass beds (Fonseca, 1994). Additionally, anthropogenic disturbances such as subsidence from ground water removal, propeller scarring from boats, thermal effects from industrial discharge, dredge and fill operations, chemical spills, wastewater discharge, and reduction in water transparency caused by increased stormwater runoff and urban development aggravate the loss of seagrass habitat (Pulich and White, 1991).

Large areas of seagrass habitat worldwide are experiencing decline (Short and Wylle-Echeverria, 1996). For example, West Bay in the Galveston Bay ecosystem held extensive areas of seagrasses, primarily *Halodule beaudettei,* in 1956. However, by 1989 no seagrass beds were found in this coastal bay (Sheridan et al., 1998; Pulich and White, 1991).

More than twenty years of reclamation studies have been conducted on the declining seagrass communities of the southeastern (Sheridan, 1992; Kenworthy and Fonseca, 1992; Sheridan and Livingston, 1983) and southwestern (Sheridan et al., 1998; Hammerstrom et al., 1998) United States. Hammerstrom et al. (1998) conducted studies on the effects of bed shape and density of transplants, transplantation methods, and container type on *Halodule beaudettei* and *Ruppia maritima* in the West Bay area of Galveston, Texas. They reported that both species can be successfully transplanted into West Galveston Bay. Early work on transplantation methods of *Zostera marina* (Phillips, 1974), *Thalassia testudinum* and *Halodule beaudettei* under artificial conditions (Fuss and Kelly, 1969) showed the need to study various factors affecting the growth and survival of transplanted seagrasses.

Additional studies on growth and survival of transplanted seagrasses include: the effect of light and its relationship with various physiological processes on *H. beaudettei* (Sheridan et al., 1998; Dunton, 1994, 1996) and *Cymodocea filiformis* (Kenworthy and Fonseca, 1996); significance of light under increased turbidity and siltation conditions (Vermaat et al., 1996); light and depth limits (Duarte, 1991); season and water depth (Tomasko and Dawes, 1990); and light and chlorophyll composition (Wiginton and McMillan, 1979). Beer (1989) studied the processes of photosynthesis and photorespiration in marine angiosperms. Pulich (1982a, 1982b, 1989) and Rosen and Webb (2000) performed experiments demonstrating that *Halodule beaudettei* requires a reduced oxidation state and organically-rich sediment. Studies have also shown that current flow is important for *Thalassia testudinum*, *Cymodocea nodosa*, (Koch, 1994) and *Zostera marina* (Fonseca and Kenworthy, 1987). The importance of sediment nutrients is also well documented by several seagrass studies (Sheridan et al., 1998; Kenworthy and Fonseca, 1992; Pulich, 1989; Short, 1987).

The importance of seagrass habitat and its decline and loss in much of the worlds’ bays and estuaries is well established in the literature. Restoration methods are still being developed and improved and commonly rely on wild donor beds. Large-scale culture of seagrasses for donor stock is an important step to supply plants for the restoration effort in a field that has a high rate of failure. Since there is a need for culturing seagrasses on a large scale, this research is developed with the intent of improving methods of pond culture and transplant techniques of seagrasses. Specific hypotheses of this research are:

1. There is no difference in the effects of reduced versus oxidized sand on the growth of *Halodule beaudettei*.
2. There is no difference in the effects of sediment media, collected from three locations, on the growth *Halodule beaudettei.*
3. There is no difference in the effect of container type and size on the growth of the seagrass *Halodule beaudettei.*
4. There is no difference in the effect of current flow regime on the growth of *Halodule beaudettei.*
5. There is no difference in the effect of light transmittance on the growth of *Halodule beaudettei*, *Ruppia maritima*, *Thalassia testudinum*, and *Halophila engelmannii*.

MATERIALS AND METHODS

Study Site

The study site was a dune and wetland plant nursery located at the Southwest corner of Pelican Island, adjacent to the Texas A&M University at Galveston (TAMUG) Mitchell campus.

Ten ponds, each measuring approximately 10 meters wide by 28 meters long and 0.3 meters deep, were used. Water was pumped from Galveston Channel into pond one by a twenty-five horsepower electric motor by way of 10.2-centimeter diameter PVC pipe. A timer was used to start the pump and water was pumped every three hours for thirty minutes beginning at midnight through 6:30 a.m. and for forty-five minutes from 9:00 am through 9:45 p.m. The water flowed into pond one at approximately 2.8 kiloliters per minute. The water flowed though each pond dike by way of culverts made from PVC pipes 20.3 centimeters in diameter. The culverts were placed at opposite ends of each pond so that water must flow from one end to the other and across each pond (Figure 1).

Water Temperature and Salinity

Water temperature and salinity were measured weekly starting in 1999 through the duration of the research. Salinity was measured in parts per thousand using an Aquafauna Bio-Marine, Inc. hand held refractometer. Temperature was measured in degrees Celsius using a hand held glass mercury or alcohol thermometer.

Transplant Units

Shoalgrass (*Halodule beaudettei*), widgeongrass (*Ruppia maritima*), star grass (*Halophila engelmannii*), and turtlegrass (*Thalassia testudinum*) were transplanted from nursery pond stock to the experimental sites in pond seven, eight, nine, or ten. Transplant units (TPUs) consisted of seagrass plants that were removed from the sediment with a gardening trowel and rinsed free of sediment. The TPUs were cut to size (one to five stems per rhizome). A trench was made using the trowel and the TPUs were placed in the trench and covered with sediment media.

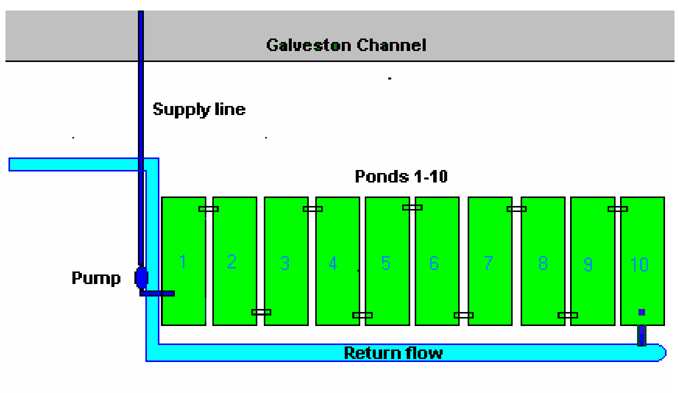

Figure 1: Nursery Pond diagram showing pond alignment, pump placement, relative location to Galveston channel, and return flow location.

Objective 1: To determine the effects of reduced versus oxidized sand on the growth of *Halodule beaudettei*.

Dry sand was placed in sixteen three-quart plastic pots. Each pot was 20.3 cm in diameter and 12.7 cm deep. In order to reduce the media, pots were placed into pond nine for two weeks prior to planting. On the day of planting (August 21, 2000), sixteen more pots were filled with dry (oxidized) sand. Shoalgrass was planted in each pot at a rate of five stems per pot. The pots were placed one after another so that the treatments formed a checker board pattern in pond number nine at a uniform depth. Data were collected on day forty-seven by counting the number of stems per pot that are visible (above the surface of the media).

Objective 2: To determine the effects of sediment media, collected from three locations, on the growth of the *Halodule beaudettei.*

Three different media sources used for this study were from Galveston Island's West Bay, East Beach ponds, and from the experimental pond bottoms. Each medium was collected wet, and if necessary transported to the ponds to prevent exposure to air and the possible oxidation of medium. Each of the three media types were placed into sixteen three quart pots. Four pots of the same media were placed into a plastic tub (68.6 cm diameter by 22.9 cm deep). Each tub, with its four pots, represented a replication. The same sediment type that was in the pots was placed around each pot in the tubs. There were four replicates for each treatment. This allowed the seagrass to grow out of the pots and into the tubs. The tubs were placed in a completely randomized block design at a uniform depth. TPUs were five stems per pot. Growth as number of stems per pot was measured at forty-eight and ninety-five days after transplant on June 27, 2000.

Medium from each source was tested for pH, nitrate, phosphorus, potassium, calcium, magnesium, salinity, sodium, and sulphur. Percent organic matter and textural analysis were also determined. All chemical analyses were performed by the Texas Agricultural Extension Services at the Texas A&M University Soil, Water and Forage Testing Laboratory, College Station, Texas. A 10.0 cm deep soil core was taken from each tub. The four samples from each treatment were combined, thoroughly mixed using hands covered with latex gloves, and placed into a clean plastic bag. This provided the lab with three samples, one from each sediment type. The samples were then placed in a box and shipped overnight to the testing lab.

Objective 3: To determine whether container type and size affects the growth of the *Halodule beaudettei.*

Two different container types were used for transplanting *H. beaudettei.* These containers were three quart capacity (20.3 cm diameter by 12.7 cm deep) plastic pots and plastic trays with eighteen wells (7.1 by 7.1 cm per well). Sixteen of each container type were used. Drain holes in the trays were covered, using mulching film, to prevent the loss of medium. The potting medium used for this experiment was collected from Galveston Island's East Beach ponds. The medium was maintained in a reduced state by transporting it wet and covered by water from the source site. Transplant units were removed as described above and planted in the pots and trays at a rate of five stems per pot, and two stems per well. Growth was measured by counting stems per pot and per well at forty-eight days and ninety-three days after transplanting on August 11, 1999.

Objective 4: To determine current flow regime on the growth of *Halodule beaudettei.*

The effect of current flow was created by placing plywood walls in pond number ten to separate the pond into current flow and non-flow areas. Transplant units with five stems were placed in the three quart pots (20.3 cm diameter by 12.7 cm deep) with reduced East Beach pond medium. Four replicates (with four pots per replicate) for each flow regime were used. Each group of four pots was placed together, and the depth of treatments with each set of replicates was measured to make sure that there was no difference between treatment depths. Growth was measured at forty-seven days after transplant as number of stems per pot. Transplanting took place on August 21, 2000.

Objective 5: To determine the effect of light transmittance on the growth of *Halodule beaudettei*, *Ruppia maritima*, *Thalassia testudinum*, and *Halophila engelmannii*.

East Beach pond sediment was collected and transported wet to the nursery pond site where it was transferred into three quart plastic pots (20.3 cm diameter by 12.7 cm deep). *Halodule beaudettei*, *Ruppia maritima*, and *Halophila engelmannii* were transplanted into the sediment at a rate of five stems per pot. *Thalassia testudinum* was planted at a rate of two stems per pot. Twelve pots of each species were divided into two treatments of six pots each. Of the two treatments, one was placed in pond seven and one in pond nine. The pots were assigned a number from a random number chart and placed accordingly in that order. The treatment in pond seven was assigned as the low light treatment and the treatment in pond nine was assigned the high light treatment. This assigning of treatments is due to the apparent fact that the ponds become clearer as the water flows from pond to pond; pond nine being clearer than pond seven. Light meters were set up in both ponds to measure photosynthetically active radiation (PAR, 400-700 nm) using paired quantum sensors (Model LI-192SA in air & Model LI-193SA submerged) with data loggers (LI-COR, Lincoln, Nebraska) during ten days of the project. Stem counts on all treatments were measured at forty-seven and ninety-four days after transplant (June 28, 2000).

Statistical Analysis

Data for the experiments was converted to percent change. Percent change is the percentage of stems rather than the raw number of stems. This number was achieved by subtracting the original number of stems from the final number of stems. The difference between these numbers was then multiplied by 100 over the original number of stems to give the percentage of new stems compared to the number of stems at the beginning of the experiment. (e.g., If a pot had five stems at the beginning and 10 stems at the end of the experiment, ((10-5) * 100/5) = 100%) there would be 100 percent growth in that pot at the end of the experiment.) The mean of all pots or wells was then used to create the mean percent change. Data was transformed using the arc sign transformation.

SAS was used to perform statistical analysis for the data collected from all of the experiments. PROC UNIVARIATE procedure was used to test experimental data for normality. A PROC GLM (Generalized Linear Model) was also performed to compare mean percent change for each treatment. (SAS Institute, 2001).

RESULTS

Nursery Pond Temperature and Salinity

From June of 1999 through October of 2000, weekly temperatures followed a seasonal pattern and ranged from 7.5 to 38^{o}C. Weekly bay and pond 8 water temperatures, for the experimental time period in 1999, and ranged from 21.5 to 37^{o}C (Figure 2). Weekly bay and pond 7, 8, 9 and 10 water temperatures, for the experimental time period in 2000, and ranged from 22 to 38^{o}C (Figure 3).

From June to September, 1999 and during mid August, 2000, some nursery pond temperatures, and at times bay water exceeded 35^{o}C. These observations are as follows: June 18, 1999 pond nine temperature was 36; July 21, 1999 pond 9 temperature was 35.5^{o}C; July 28, 1999 the bay and ponds 7, 8, 9, and 10 were 36, 36, 36, 36, 37, and 36^{o}C, respectively; August 6, 1999 the bay and ponds 7, 8, 9, and 10 were 36, 37, 37, 37, 38, and 37.5^{o}C, respectively; August 24, 1999 ponds 8, 9, and 10 were 36, 35.5, and 36.5^{o}C, respectively; September 1, 1999 ponds 9 and 10 were 37 and 36^{o}C, respectively; August 7, 2000 ponds 7, 8, and 10 were 36, 35.5, 38^{o}C, respectively; and August 14, 2000 ponds 8 and 9 were 36 and 37.5^{o}C.

From June of 1999 through October of 2000 salinity ranged from 20 to 41 parts per thousand (ppt) (Figure 4). High salinity values were typical during periods of low rainfall, and low salinity values were typical during periods of high rainfall. Weekly bay and pond 8 water temperatures, for the experimental time period in 1999, are shown in Figure 4 and ranged from 21.5 to 37^{o}C. Weekly bay and pond 7, 8, 9 and 10 water temperatures, for the experimental time period in 2000, and ranged from 22 to 38^{o}C (Figure 5).

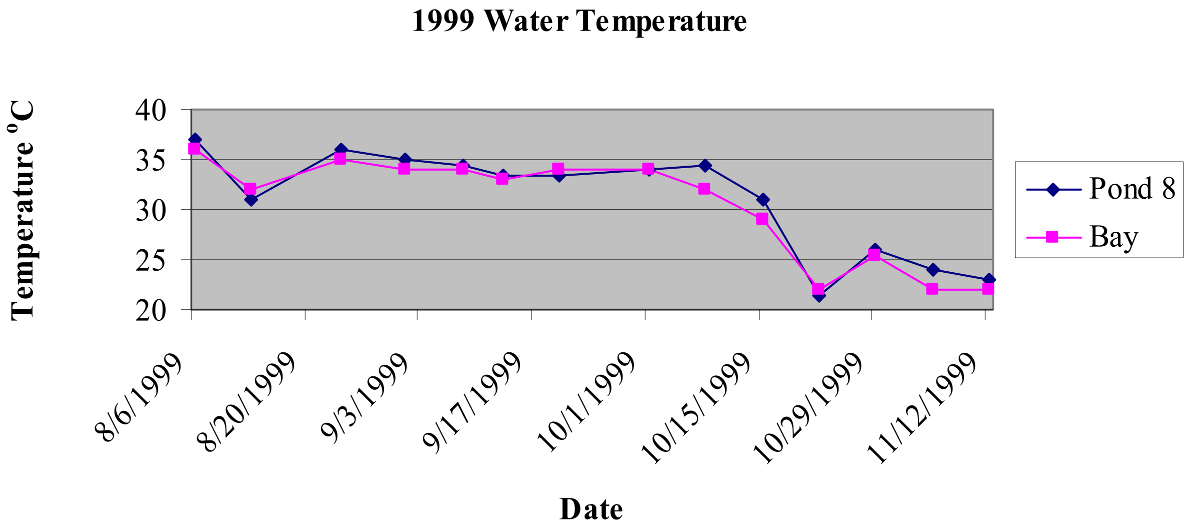

Figure 2: Weekly temperature for bay water and pond 8 during experimental time frame from August 6, 1999 to November 12, 1999.

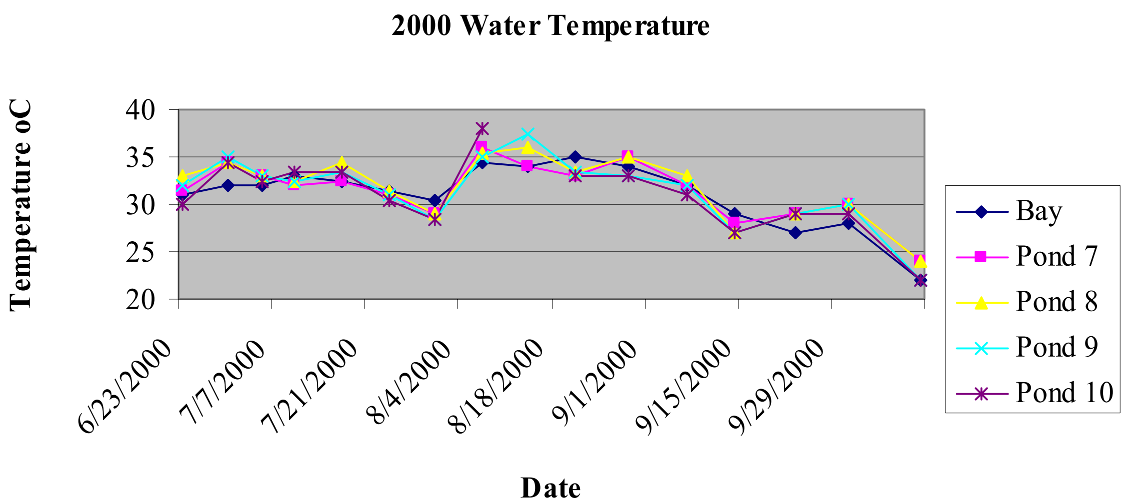

Figure 3: Weekly temperature for bay water and pond 7, 8, 9, and 10 during experimental time frame from June 23, 2000 to October 12, 2000.

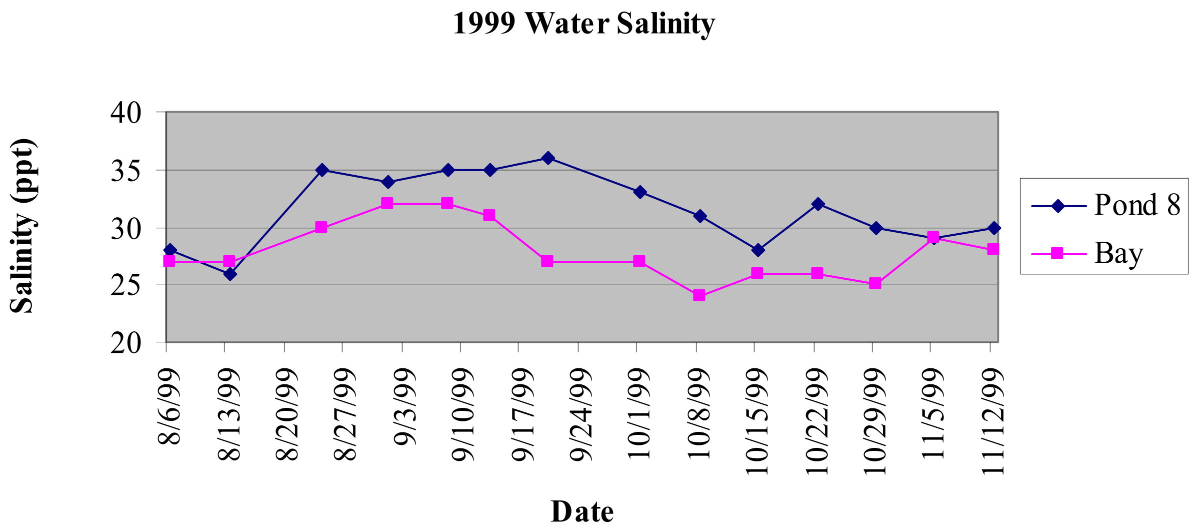

Figure 4: Weekly salinity for bay water and pond 8 during experimental time frame from August 6, 1999 to November 12, 1999.

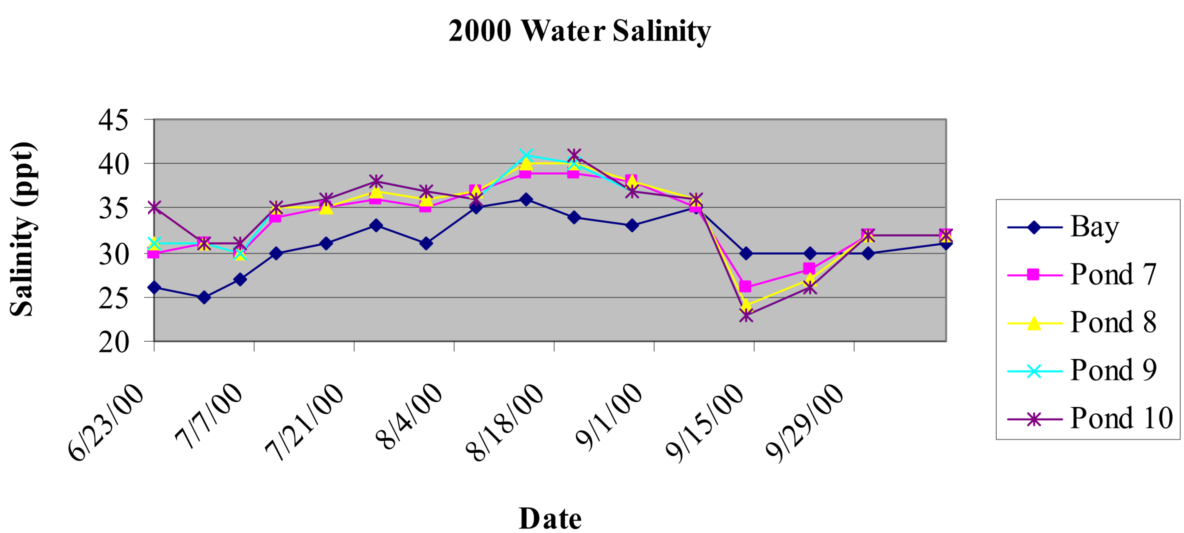

Figure 5: Weekly salinity for bay water and pond 7, 8, 9, and 10 during experimental time frame from June 23, 2000 to October 12, 2000.

Experiment 1: To determine the effects of reduced versus oxidized sand on the growth of *Halodule beaudettei*.

Experiments to determine the effects of reduced and oxidized sand were run for 47 days starting August 21, 2000. The results of the oxidation/reduction experiment show that there is no significant difference in the mean percent change in the number of stems between transplants in oxidized (76.3 % ± 12.8% standard error of the mean) and reduced (73.8% ± 12.1%) sand after 47 days ($p = 0.01$) (Figure 6). The total mean number of stems in oxidized and reduced sand was 8.8 and 8.7 stems, respectively. Tests conducted to determine normality of the data using Shapiro-Wilk test revealed that the data has normal distribution ($p > 0.05$).

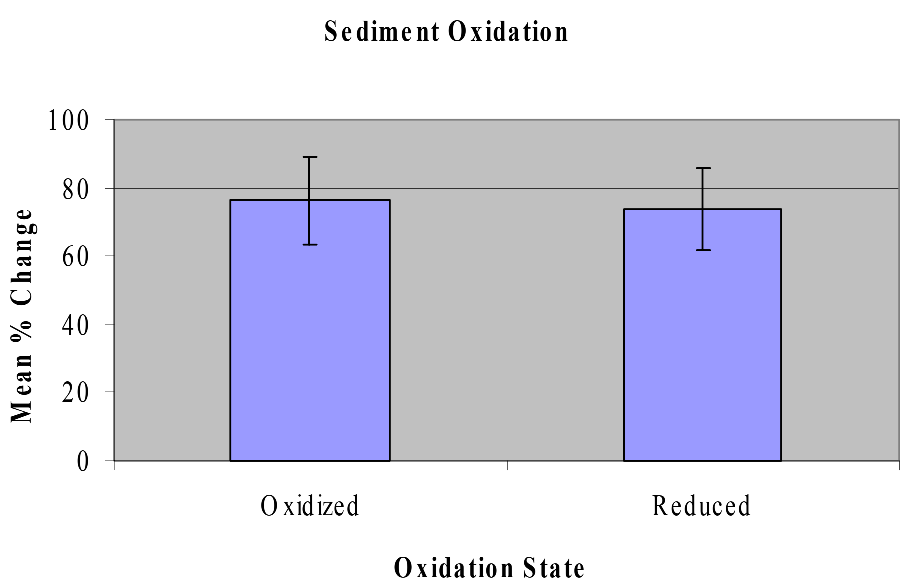

Figure 6. The mean percent change in the number of stems for *Halodule* in oxidized sand versus reduced sand after 47 days. Error bar represents ± the standard error of the mean.

Experiment 2: To determine the effects of sediment media collected from three locations, on the growth of the *Halodule beaudettei.*

Experiments to determine the effects of sediment media were run for 48 and 95 days beginning on June 27, 2000. The effect of sediment types on the growth of *Halodule* at 48 days after planting showed that there were no significant differences ($p = 0.51$) among sediment from West Bay, East Beach lagoons, and the experimental pond bottom. Tests conducted to determine normality of the data using Shapiro-Wilk test revealed that the data has normal distribution ($p > 0.05$).

The mean percent change in the growth of *Halodule* was 308.8 ± 50.5 in sediment from West Bay, 275.0 ± 41.6 in sediment from East Beach, and 217.5 ± 71.8 in sediment from the experimental pond bottom (Figure 7). At 48 days after transplanting, there where 20.4 stems per pot in West Bay sediment, 18.8 stems per pot in East Beach sediment, and 15.9 stem per pot in the experimental pond bottom sediment.

The effect of sediment types at 95 days after planting showed that there were no significant differences ($p = 0.57$) among sediments from West Bay, East Beach, and the experimental pond bottom. At 95 days after transplanting, the mean percent change in the growth of *Halodule* was 1,555.0 ± 177.9 in sediment from West Bay, 1,425.0 ± 165.3 in sediment from East Beach, and 1,247.5 ± 254.9 in sediment from the experimental pond bottom (Figure 8). This growth produced a total mean number of 82.8 stems per pot in West Bay sediment, 76.3 stems per pot in East Beach sediment, and 67.4 stem per pot in the experimental pond bottom sediment.

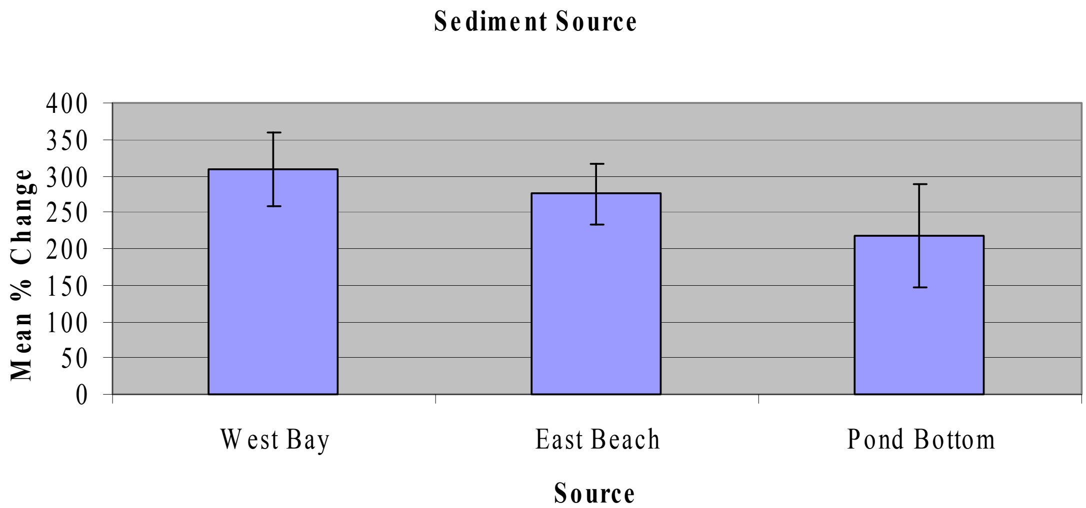

Figure 7. Mean percent change in growth of *Halodule* at 48 days in sediment from West Bay, East Beach, and the experimental pond bottom. Error bar represents ± the standard error of the mean.

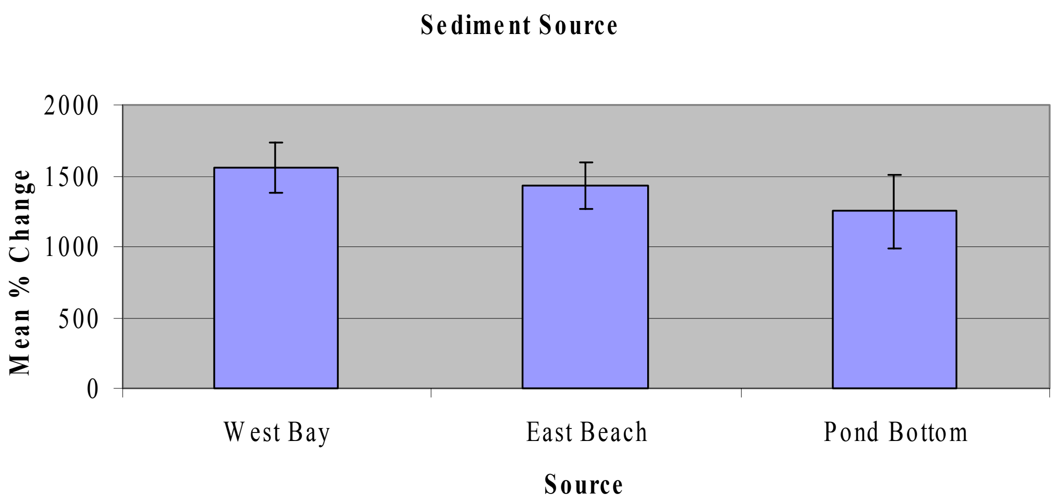

Figure 8. Mean percent change in growth of *Halodule* at 95 days in sediment from West Bay, East Beach, and the experimental pond bottom. Error bar represents ± the standard error of the mean.

Experiment 3: To determine whether container type and size affects the growth of the *Halodule beaudettei.*

Experiments to determine the effects of container type were run for 48 and 93 days beginning on August 11, 1999. The effect of container types at 48 days after planting showed that there was a significant difference ($p = 0.02$) between pots and trays. The mean percent change in the growth of *Halodule* was 840.0 ± 144.8 in trays and 425.3 ± 79.5 in pots (Figure 9). Tests conducted to determine normality of the data using Anderson-Darling test revealed that the data has normal distribution ($p > 0.05$).

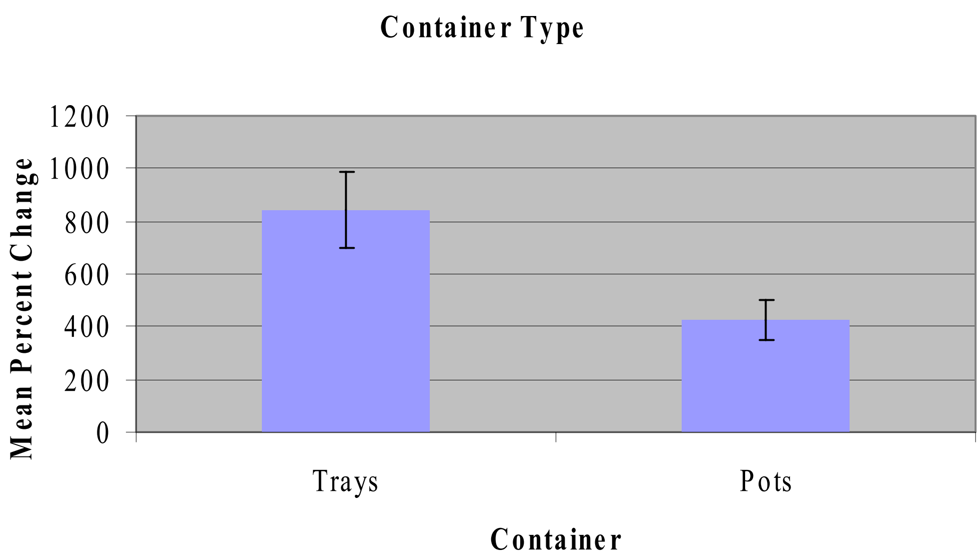

Figure 9. Mean percent change in the number of stems produced at 48 days in trays and pots. Error bar represents ± the standard error of the mean.

The effect of container types at 93 days after planting showed that there was not a significant difference ($p = 0.82$) between pots and trays. The mean percent change in the growth of *Halodule* was 996.8 ± 172.3 in trays and 942.7 ± 156.9 in pots (Figure 10). Tests conducted to determine normality of the data using Anderson-Darling test revealed that the data has normal distribution ($p > 0.05$).

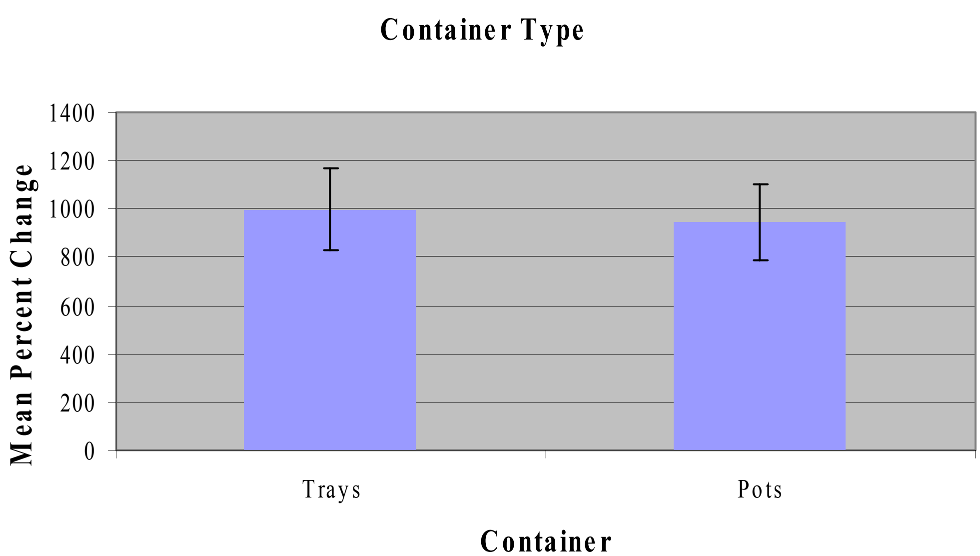

Figure 10. Mean percent change in the number of stems produced at 93 days in pots and trays. Error bar represents ± the standard error of the mean.

Experiment 4: To determine current flow regime effects on the growth of *Halodule beaudettei.*

Results for the effect of flow of water versus no-flow of water on the location of pots in pond 10 show that there was no significant interaction ($p < 0.05$) between location of the pots and the flow regime on percent stem growth. The main effect of flow versus no-flow on the mean percent stem growth was found to be significant ($p < 0.05$). Tests conducted to determine normality of the data using Shapiro-Wilk test revealed that the data has normal distribution ($p > 0.05$).

The initial count for each plant was five stems per sprig on the date of planting, August 21, 2000. At 47 days after transplant, the effect of water flow on the growth of *Halodule* showed a mean percent change in the number of stems was 300.0 ± 54.70 for the flow treatment and 137.5 ± 47.6 for the no-flow treatment (Figure 11). The total mean number of stems recorded was 20.0 stems per pot in the flow treatment and 11.9 stems per pot in the no-flow treatment.

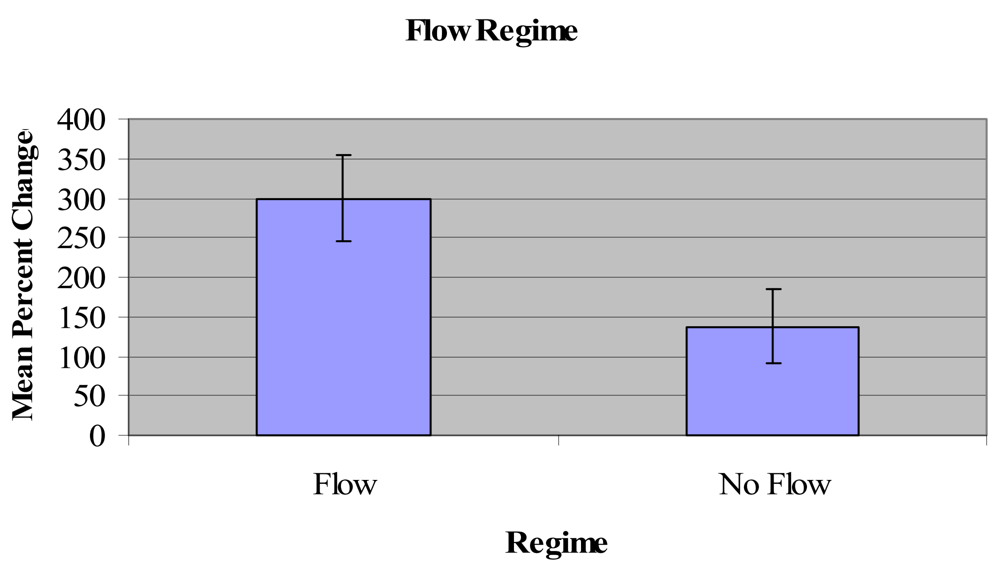

Figure 11. Mean percent change in the number of stems in the flow and no-flow treatments. Error bar represents ± the standard error of the mean.

Experiment 5: To determine the effect of light transmittance on the growth of *Halodule beaudettei*, *Ruppia maritima*, *Thalassia testudinum*, and *Halophila engelmannii*.

Direct observations show that less light was transmitted through the water in pond 7 relative to pond 9. A Secchi disk showed a difference in water clarity. The disk could not be seen at the level of the plants in pond 7, but could be seen at the level of the plants in pond 9.

Experiments to determine the effect of light transmittance for 47 and 94 days were started on June 28, 2000. Studies conducted on the effects of light transmission on the mean percent growth of stems produced significant results.

At 47 days after planting there was a significant difference ($p = 0.0003$) in the production of *Halodule* in pond 7 versus pond 9. Similarly, at 94 days after planting there was a significant difference ($p = 0.0042$) in the mean percent change of stems in pond 7 verses pond 9. The mean percent change in the number of stems in pond 7 at 47 days was -86.7 ± 9.9, and in pond 9 was 60.0 ± 25.8. The mean percent change in the number of stems in pond 7 at 94 days was -100.0 ± 0, and in pond 9 was 583.3 ± 185.6 (Figure 12).

At 47 days after planting there was a significant difference ($p = 0.0003$) in the production of *Ruppia* in pond 7 versus pond 9. The mean percent change in the number of stems in pond 7 at 47 days was -100.0 ± 0, and in pond 9 was 30.0 ± 37.2. At 94 days after planting there was a significant difference ($p = 0.049$) in the mean percent change of stems in pond 7 versus 9. The mean percent change in the number of stems in pond 7 at 94 days was -100.0 ± 0, and in pond 9 was 170.0 ± 120.8 (Figure 13).

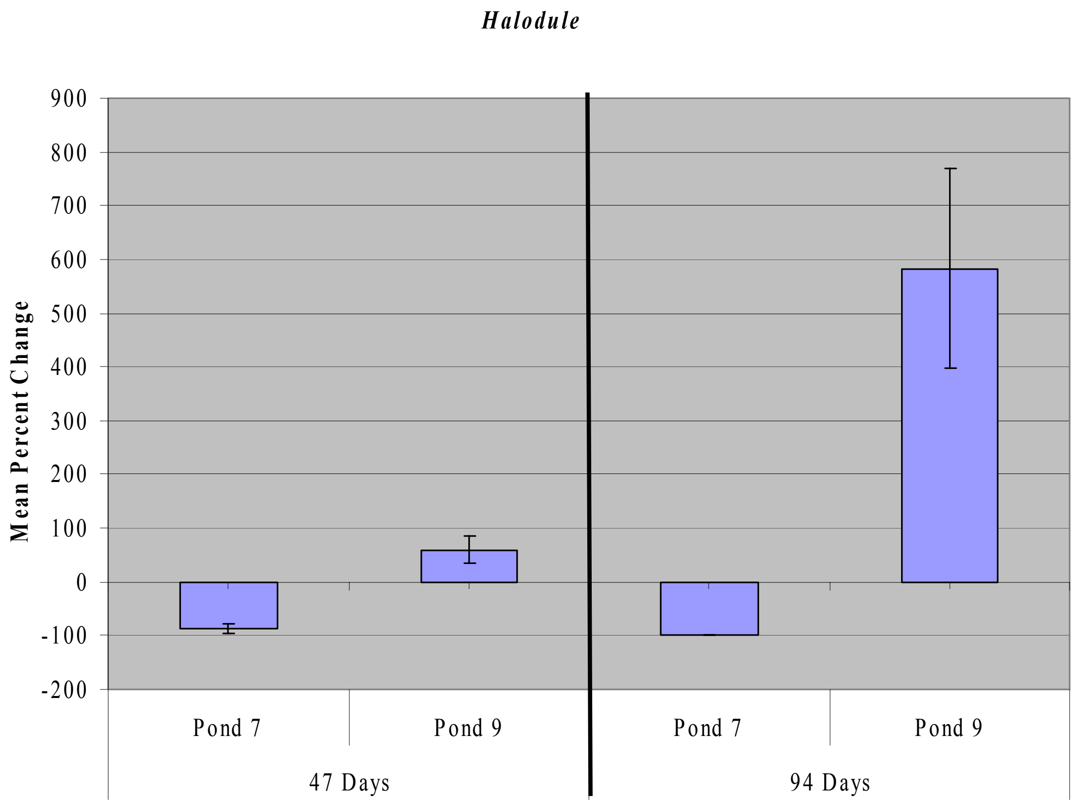

Figure 12. Mean percent change in the number of stems at 47 and 94 days for ponds 7 and 9 for *Halodule*. Error bar represents ± the standard error of the mean.

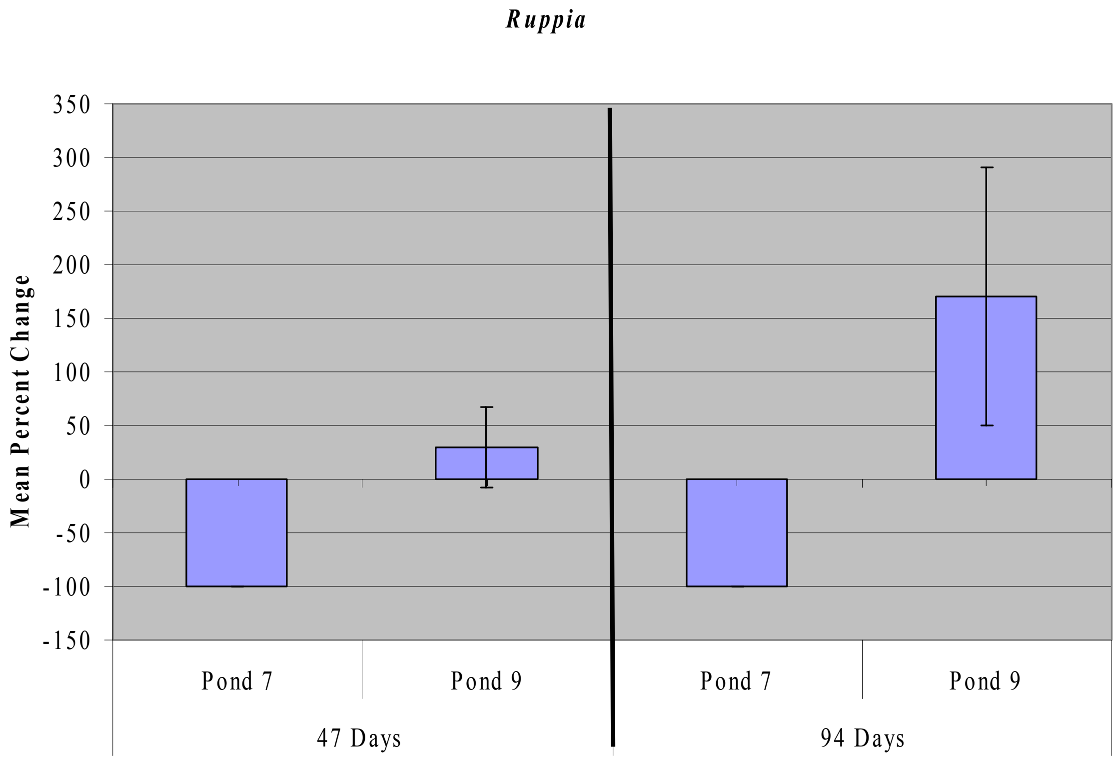

Figure 13. Mean percent change in the number of stems at 47 and 94 days for ponds 7 and 9 for *Ruppia*. Error bar represents ± the standard error of the mean.

At 47 days after planting there was a significant difference ($p = 0.0012$) in the production of *Halophila* in pond 7 versus pond 9. At 94 days after planting there was a significant difference ($p = 0.0002$) in the mean percent change of stems in pond 7 versus 9. The mean percent change in the number of stems in pond 7 at 47 days was -100.0 ± 0, and in pond 9 was 43.3 ± 32.0. The mean percent change in the number of stems in pond 7 at 94 days was -100.0 ± 0, and in pond 9 was 0.0 ± 17.1 (Figure 14).

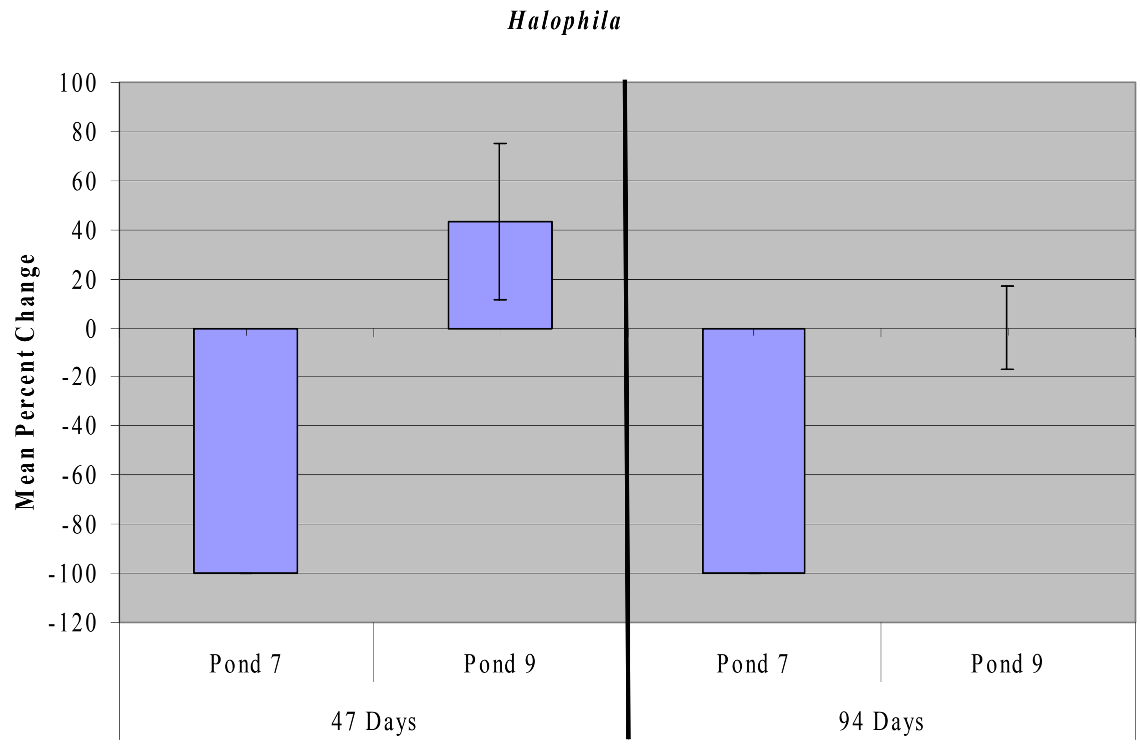

Figure 14. Mean percent change in the number of stems at 47 and 94 days for ponds 7 and 9 for *Halophila*. Error bar represents ± the standard error of the mean.

At 47 days after planting there was a significant difference (p = 0.0004) in the production of *Thalassia* in pond 7 versus pond 9. At 94 days after planting there was a significant difference (p = 0.0001) in the mean percent change of stems in pond 7 and in pond 9. The mean percent change in the number of stems in pond 7 at 47 days was -8.3 ± 15.4, and in pond 9 was 141.7 ± 20.1. The mean percent change in the number of stems in pond 7 at 94 days was -16.7 ± 30.7, and in pond 9 was 341.7 ± 50.7 (Figure 14).

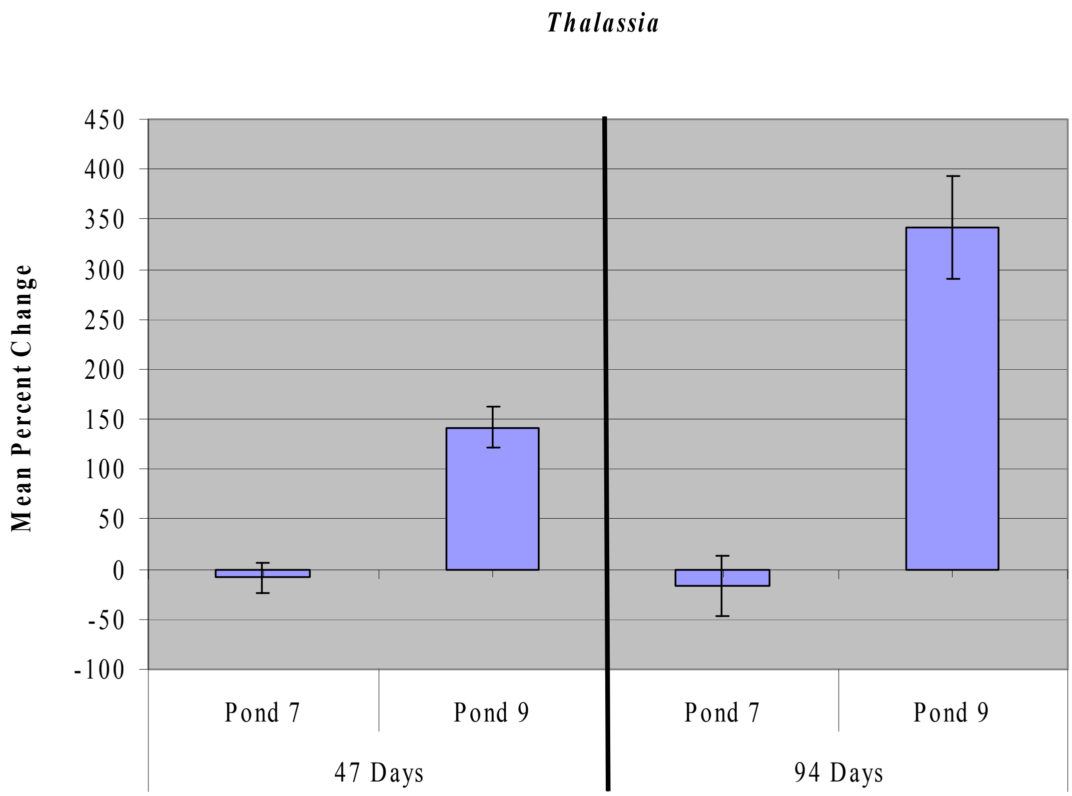

Figure 15. Mean percent change in the number of stems at 47 and 94 days for ponds 7 and 9 for *Thalassia*. Error bar represents ± the standard error of the mean.

The light in pond 7 was less than the light in pond 9 due to the sediment load in pond seven. The data retrieved from the light meters was not usable, and thus, is not presented in this thesis. However, direct observation lead to the obvious conclusion that less light is transmitted through the water in pond 7 relative to pond 9. A Secchi disk showed a difference in water clarity between pond 7 and pond 9. The disk could not be seen at the level of the plants in pond 7, but could be seen at the level of the plants in pond 9.

DISCUSSION

Nursery Pond Temperature and Salinity

From June to September, 1999 and during mid August, 2000, some nursery pond temperatures, and at times bay water, exceeded the tolerance limits of 2 to 35^{o}C for *Halodule beaudettei* as suggested by McMillan (1979). These high temperatures may have affected experiments on the effect of light, sediment source, and container type. The experiments on sediment oxidation state and flow regime did not fall in the time frame when temperatures exceeded 35^{o}C. Salinity levels in the bay and in the experimental ponds were within the tolerance limits of 3.5 to 70 ppt for *Halodule beaudettei* as defined by McMahan (1968).

Experiment 1: Effect of reduced versus oxidized sand on the growth of *Halodule beaudettei*.

The media oxidation state at planting did not affect the growth of shoal-grass. Other experiments and observations (Pulich 1982a, 1982b, 1989) led us to question whether an oxidized medium would inhibit growth of shoal-grass when compared to the growth of shoal-grass in a reduced medium. Rosen and Webb (2000) noted a difference in growth when using an oxidized medium versus a reduced medium. However, the media they used were of different origins; dry sand (from the experimental nursery site), shell fragments (from the experimental nursery site), and highly reduced marine sediment (from the East Beach Lagoons). These media also differed in sulfide concentrations and nutrient levels (Rosen and Webb 2000). The reduced medium we used was dry sand (from the experimental nursery site) placed in pots and transferred to the experimental ponds for reduction two weeks before transplant. Our oxidized medium was the same sand from the same source as the reduced medium. Since soil reduction occurs rapidly with submersion, we wanted to confirm or disprove an increase in growth when reduced media is used. Interestingly, at similar sampling periods, Rosen (1998) found either no growth or even a loss in the number of stems in oxidized media whereas, we found approximately the same growth in oxidized and the reduced media. Our data suggests that the relationship of media oxidation state at planting may not be as important as the nutrient makeup of the media.

Further data to support this position is found in our Experiment 2 (Effect of sediment media, collected from three locations, on the growth of *Halodule beaudettei*). Data from this experiment show the medium from East Beach, the same media source Rosen used for his reduced medium (personal communication) (Rosen, 2001), had a higher mean growth than that in of our reduce or oxidized sand media treatments. We believe this difference is due to the higher nutrient level of sediment from East Beach and not from the lower redox potential.

Another difference between Rosen's experiment and our experiment was the time of year. Rosen started his experiment in April and we started ours in August. However, this difference does not account for the near equal growth of *Halodule* in our experiment. Temperature and salinity during the time period of this experiment were within the tolerance limits of *Halodule* as they were in the Rosen's experiment.

Experiment 2: Effect of sediment media, collected from three locations, on the growth of *Halodule beaudettei*.

The source of growth medium did not significantly affect the growth of shoal-grass. Sheridan (personal communication, 2000) found areas of West Bay, Galveston, Texas unsuitable for growing shoal-grass, and he suggested that the sediment at this location might be unsuitable for its growth. We compared three media types to test his assumption. Sediments were taken from West Bay, East Beach, and from the experimental pond bottom. No significant differences were found in the growth of shoal-grass between these three sediment types. Therefore, we conclude that it is likely that other factors are affecting the growth of shoal-grass in areas of West Bay. During visits to West Bay for sampling, large numbers of bioturbators were seen (blue crabs and stingrays). In addition, Rosen (1998) reported human disturbances of the area, likely caused by local boaters and fishermen. At other times, we observed shoal-grass floating at West Bay. This suggests two points of interest. One, it is likely that something was uprooting the shoal grass; and two, shoal grass is growing in West Bay of the Galveston Bay Ecosystem, Texas.

Experiment 3: Effect of container type and size on the growth of *Halodule beaudettei*.

In an effort to determine the preference of container type used for the growth of shoal-grass transplants for planting into the wild, plastic pots were compared with plastic trays. The container type was significant at 48 days and shoal-grass produced a significantly higher number of stems in trays than in pots. However, by 93 days, there was no significant difference between the two container types.

The premise behind the experiment was to determine which container type made the better transplant container for the growth of shoal-grass for transplant into the natural ecosystem. If a seagrass nursery had a short (48 day) time frame in which to grow shoal-grass, they may want to choose trays over pots. But if the time frame for use of the shoal-grass was longer, they may want to evaluate other factors. Since the trays are easier to rip apart, they appear to be easier to work with, however, whole trays tend to bend and twist during transport by hand thus, making it less efficient. Likewise, the pots were three quarts full of sediment, making them bulky and heavy. Since plastic can not be legally placed in the marine environment, further experiments may be needed to study the effects of different container types such as peat pots. Experiment sampling periods may also need to be shortened or lengthened depending on container size.

Experiment 4: Effect of flow regime effects on the growth of *Halodule beaudettei*.

Flow regime has an effect on the growth of shoal-grass. The shoal-grass in the flow area had a greater number of stems per pot than the shoal-grass in the no-flow area. Rosen (1998) did not find a difference with higher rates of flow versus lower rates of flow, and Fuss and Kelly (1969) did not show that continuous circulation in tanks to be beneficial, but personal conversations with other scientists led us to believe that some flow is a necessary part of seagrass and SAV culture. Although a number of experiments (Fuss and Kelly, 1969; Fonseca and Kenworthy, 1987; Koch, 1994; Rosen 1998) have shown a variety of results for current and flow, it has been our experience that current, or flow of water, is an essential part of seagrass growth. It has yet to be determined what level of flow is necessary and whether the difference is caused by dissolved oxygen/carbon dioxide, nutrient flow, or other parameters.

Water exchange occurred in the no-flow area due to gaps between the plywood dividers and substrate bottom, but direct flow or current was limited by the dividers and the surrounding emergent grass (*Spartina alterniflora*). Flow was not measured due to the low rates of flow, even in the flow area. However, flow could be seen as water leaving the ponds and debris floating in the ponds.

Care was taken to reduce certain confounding factors (turbidity, depth, and algae growth). All sets and subsets were grown within pond number ten. Pond ten was the last and the clearest pond in the series. This method was designed to eliminate turbidity as a factor affecting the growth of *Halodule*. Care was taken to put all experimental sets and subsets at the same depths within the pond. As the pond bottom was uneven, bricks and cinderblocks were used to adjust height as needed. The growth of algae, which has been reported to interfere in seagrass growth in various other studies (Sand-Jensen, 1977; Dunton, 1990; Dunton 1996; Rosen 1998), was only limited in this study by keeping the experiment time to 47 days. No physical attempt was made to limit algae growth.

Experiment 5: Effect of light transmittance on the growth of *Halodule beaudettei*, *Ruppia maritima*, *Thalassia testudinum*, and *Halophila engelmannii*.

Light transmittance had an affect on the growth of the various SAVs. *Halodule*, *Ruppia*, and *Halophila* did not survive beyond 90 days after transplanting in pond 7 (at the lower light levels) and *Thalassia* had only minimal growth by 90 days (After starting at 2 stems per pot, Thalassia ended with a mean of 4.8 stems per pot.) In pond 9, however, all SAVs had increased mean number of stems per pot by 45 days (in the higher light levels). Further, all of the SAVs except *Halophila* had a greater mean number of stems per pot by 90 days in pond 9.

Since algae was observed to be growing on all plants in pond 9, it is likely that SAV growth was limited by the algae, effectively reducing the light available to the plant. It is possible that *Halophila* could not tolerate the stress caused by the algae and thus, died back between 47 and 90 days. Epiphytic algae were not a problem in pond 7 due to the lower light levels. Limiting algae in pond 9 was attempted by removing floating algae. Floating algae could limit light, and was removed by scooping it out of the pond. Care was taken not to enter the ponds during experiments as this action could disturb the pond bottom and cause degraded water clarity. Monitoring flow rates also helped reduce algae accumulation on the water surface, as higher currents would keep some surface algae washed out of the ponds.

The light in pond 7 was less than the light in pond 9 due to the sediment load in pond seven. A Secchi disk showed a difference in water clarity between pond 7 and pond 9. However, this measurement does not accurately quantify the difference as well as light meters would have.

It is difficult to compare certain experiments. For example, the sediment study (Experiment 2) and light study (Experiment 5) were setup within one day of each other, June 27 and June 28, 2000, both used pond 9, and both used sediment from East Beach, at least in some treatments. However, at 48 and 47 days, these treatments are not similar in growth. The sediment study, with East Beach lagoon sediment, had a mean percent change of 275.0 and the light study had a mean percent change of 60.0. Since some treatments in these experiments where grown in the same sediment, pond, container type, and within the same time frame, it is difficult to speculate about the difference in growth. Two differences are the depth of the containers and the location within the pond, affecting the temperature and the amount of flow these experiments received. Ironically, the light experiment was in deeper water than the sediment experiment and would thereby have received less light, but would likely have experienced lower temperatures. Since light is known to affect seagrasses, this may be the limiting factor showing the difference in growth between the experiments.

The redox experiment (Experiment 1), with a mean percent change of 73.8 in the reduced medium, and the sediment experiment, with a mean percent change of 275.0 in the East Beach lagoon sediment, are easier to compare. Both of these experiments took place in pond 9 using the same container type. However, neither had the same sediment source, start time, depth, or position within the pond. It is easy to speculate that the media difference between the experiments was the limiting factor.

The flow experiment (Experiment 4) and the sediment experiment had similar mean percent changes in growth. The flow treatment in pond 10 had a mean percent change of 300.0, and the East Beach sediment treatment had a mean percent change of 275.0. Both of these experiments were run in sediment from East Beach lagoons and in the same container type. They differed in that the flow experiment took place in pond 10, the sediment experiment took place in pond 9, and they were started almost one month apart.

Since the growth of *Halodule* in the redox experiment and pond nine in the light experiments have lower percent mean growth, they compare well. The results of the sediment, container (Experiment 3), and flow experiments also compare well, each with higher percent mean growth. The number of variables involved in each experiment confounds comparisons. Clearly, more experimentation is needed to fully assess seagrass culture techniques.

The cultivation of SAVs in a nursery pond complex was improved by experiments designed to study the effects of media oxidation state, media source, container type, flow regime, and light transmittance. Constant care was needed in order to maintain the seagrass nursery pond complex. Daily visits to the site were necessary during the growing seasons. The main purpose for the daily visits was to check the water circulation to determine that the pumps where working. Weekly hydrographic parameters where checked and pump maintenance was performed. Pump maintenance included checking and replacing sacrificial anodes and greasing the pump. Occasionally the pump intake from the bay was checked for blockages and fouling and intake pipes where checked for leaks.

Even after extensive pump maintenance was performed the pump would occasionally break down. At these times it was necessary to rent backup pumps. The backup pumps where diesel powered and required refueling and manual starts and stops. Depending on the pump that was available for rent at the time of the malfunction, the pumps varied in horsepower and flow output. Although the pump speed could be set by throttling down the engine, all rental pumps had a greater output flow rate than the electric pump, even at it’s slowest maintainable flow rate.

Epiphytic and free floating algae were also a maintenance problem during the peak of the growing season. Free floating algae were removed from the water surface by scooping it out with nets from the pond dikes. Algae might better be controlled in concrete ponds. Concrete ponds might also better control turbidity since at least some amount of turbidity was created by water falling into the ponds from the pump station.

CONCLUSIONS

Seagrass beds are an important natural ecosystem that may be helped by compensatory mitigation for lost or damaged sites, construction, and restoration. Nursery pond cultivated seagrasses are a potential replacement source for donor beds used in restoration activities.

The nursery pond cultivation of seagrasses and SAVs can be improved through the careful study and experimentation of cultivation methods and practices. The media oxidation state at the time of transplant of *Halodule* appears to have no effect upon the production of new stems of this seagrass, however these results differ from previous research. Since chemical reduction of the growth media occurs very rapidly upon submersion, it is unlikely that it would be a limiting factor for seagrass production. It is likely that media and water nutrient levels play a larger role than media oxidation state.

The media from West Bay did not appear to limit *Halodule* stem production when it was compared to the stem production of *Halodule* grown in media from East Beach or the experimental pond bottom. It is unlikely that West Bay is unsuitable for *Halodule* growth based on the composition of the bay sediment.

The container types used in this study worked well for the nursery pond cultivation of SAVs. However, due to laws and regulations, plastic is not suitable for use in the marine environment. Further studies to determine the best container type are warranted, and it may be necessary to use a combination of peat pots in plastic containers for nursery stock that is to be transplanted into the natural environment.

Our studies and conversations with other scientists show that some type of current or flow is important in the production of SAVs. The exact reason for this has yet to be determined however, the limiting factor caused by very low flow water may be dissolved gas exchange or water column nutrient exchange.

Our studies show that limited light caused by sediment turbidity has a negative impact on the production of SAV transplants. Since light may be limited by sediment loading of natural ecosystems, further experiments to show the light limiting threshold of SAVs may be important in understanding the success of transplants into the natural environment.

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